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PHOTOREACTIVATION OF EXCYSTMENT DELAY IN COLPODA INDUCED BY FAR ULTRAVIOLET RADIATION

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Running title: Photoreactivation in cysts

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No Tables

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SUMMARY

1. No photoreactivation (PR) was observed in dry cysts of Colpoda inflata irradiated with either monochromatic 265 nm far UV radiation or with polychromatic radiation from an argon plasma arc lamp filtered through a sapphire window with a cutoff at 145 nm.

2. Wet Colpoda cysts, however, showed a high degree of PR, especially after large UV doses from either source listed above. PR was achieved using two lamps, each with two 4 watt Cool white fluorescent bulbs at 8 cm from the cyst preparation; an exposure of 4 or more hours was required for maximal PR.

3. Wet cysts were damaged by much smaller doses of far UV than dry cysts; such damage is, however, also subject to PR.

4. It is probable that PR fails in dry cysts because the photoenzyme which provides energy for PR by absorbing visible and near UV radiation is unable to act in the absence of water.

Photoreactivation (PR) of effects of far ultraviolet (UV) on cells by visible and near UV radiation is believed to consist mainly, if not entirely, of monomerization of pyrimidine dimers catalyzed by a photoenzyme which absorbs the visible and near UV radiation. The photoenzyme is widely distributed in plants, animals and microorganisms but is absent from placental mammals [4]. Since resting cysts of ciliate protozoans, wet or dry, show almost imperceptible oxygen consumption [13, 15] and many of the hydrolytic and oxidative enzymes of even wet cysts are much less active than in vegetative cells [14], one might expect them to be incapable of PR. Tests reported here, however, indicate that under certain conditions the damaging effect of far UV on cysts is susceptible to PR.

MATERIALS AND METHODS

The colpodas were grown in the manner already described [10] in a salt medium on a suspension of bacteria (Pseudomonas ovalis). The species used in this study (Colpoda inflata) is larger than C. steinii used for radiation studies previously [8] and more resistant to far UV radiation than the latter; therefore, larger doses of UV radiation were used in the present study than previously.

Wavelength 265 nm (obtained from a quartz mercury arc and quartz monochromator), the intensity of which was determined with a thermopile calibrated against standard lamps, was used for irradiation in most of the experiments because it strongly retards excystment of Colpoda [8]. The Colpoda cysts, held on ca. 3 x 3 mm squares of cellophane cut from a larger sheet on which encystment had been induced (according to a modification of the method previously used), were placed face downward in a quartz cell for irradiation,

with the UV coming from below. The intensity of the radiation declined with use of the arc from about 35 to about 19 ergs $mm^{-2}sec^{-1}$. In a few experiments cysts were exposed to wavelength 320 nm because it represents far UV radiation prominent in sunlight UV at the surface of the earth. In still other experiments cysts were exposed to radiation of the entire spectrum of an argon plasma arc lamp transmitted through a sapphire window (145 nm cutoff), the radiation span of which simulates non-ionizing space radiation, but of higher intensity, as described elsewhere [10].

For PR the preparations, face up, were exposed for approximately 4 to 12 hours at 8 cm from two Yale and Stocker Lite Mite lamps, each containing two 4 watt cool white fluorescent bulbs (approximately 400 footcandles). The light was filtered through two 1 cm water cells to remove heat (the temperature in the lower cell did not rise above 26°C). The controls kept in darkness were periodically observed at the same time as those being tested for PR through Corning filter #3484 which removes photoreactivating wavelengths. For each experiment a cellophane preparation of cysts was cut into three parts: one piece was irradiated and then placed in darkness, a second piece was irradiated and then placed in light to test for PR, and a third piece served as control. Earlier studies showed that fluorescent light of itself had no measurable effect on unirradiated cysts. Thus the cysts for a particular test had been subjected to the same microhabitat right from the time of encystment until exposure to radiation. Each cyst preparation for a test contained from about 100 to 450 cysts, mostly about 200. At least three tests were made for each dose; more were made when the data were inconsistent. Only data from one illustrative experiment are given in the figures because the relative values are of moment here, not the absolute ones. The relative positions of the curves were the same in all of the experiments of a series.

For determination of excystment time, the cyst preparations were placed face upwards in 1% sterile yeast extract in a moist-chambered Columbia watch glass and observed at approximately hourly intervals for controls and at longer intervals for the irradiated samples, the interval increasing with the dose. A final reading of the total per cent excystment was made approximately 24 hours after the preparations were placed in excystment medium. The time for 50% excystment was determined by plotting the per cent of Colpoda excysted against time.

RESULTS

1. Relative far-UV sensitivity of wet and dry cysts

A given dose of far UV radiation is far more effective on wet than on dry cysts (Fig. 1). Immersion of cysts in aqueous solution results in their immediate wetting, causing much less scattering of the incident UV radiation; consequently, more of the incident UV reaches the internal structures of the cysts. Therefore, the cysts appear more vulnerable but are receiving more UV radiation. However, it is also possible that hydration alters the vulnerability of the cysts to UV in other ways besides increasing their physical permeability to UV by wetting.

Thus cysts which have been hydrated for a few hours before the addition of excystment medium always excyst earlier than those which have been kept dry up to the time of addition of excystment medium. In fact, occasionally cells may even excyst in salt solution before the addition of excystment medium. This may be a result of hydration alone but more likely it is produced by the leaching from the cyst preparation of traces of excystment exciting substances into the salt solution. In any event, whatever the mechanism, hydration appears to partially activate the cysts. It is likely that no enzymes are active while dry but may become active at even a low relative humidity [16].

With increasing UV doses excystment is retarded to increasing degrees and the per cent excystment decreases. This corresponds to what is observed for dry cysts except that the amount of irradiation required for a given effect is less.

2. Tests for photoreactivation in dry and wet cysts

To keep the cysts dry during rainy weather, cysts which had been irradiated dry with far UV 265 mm doses of 25,000, 50,000, 75,000 and 100,000 ergs mm^{-2} were placed in ground glass-stoppered, grease-lubricated stender dishes over $CaCl_2$ in one series, and over colored Dririte ($CaSO_4$) in another, for 6 to 18 hours before exposure to visible light. Previous experiments had shown that there is no dark recovery in dry cysts [10], therefore the 6 to 18 hour delay before testing for PR does not invalidate the experiments. A similar series of experiments were performed with cysts dried in the same manner but exposed to unfiltered argon lamp radiations (simulating space radiation) for 20 seconds in one series and 30 seconds in another series of experiments, and then subjected to visible light. In no case was there evidence of PR.

In the next series of experiments the cysts were immersed in balanced salt solution and irradiated with far UV 265 mm doses of 6250, 12,500 and 25,000 ergs mm^{-2} to induce progressive retardation of excystment. The preparations were then illuminated with fluorescent light (see Methods) for 4 to 12 hours (illumination for less than 4 hours failed to give maximal PR) and unequivocal evidence for PR was obtained (Fig. 2). PR was most marked when the UV dose was greatest, as is generally found to be the case. In all cases PR was a dose reduction, that is, the cysts acted as if they had received a lesser dose of radiation but they did not recover completely from the effects of far UV radiation.

A few experiments were also performed with far UV 302 nm which is prominent in sunlight at the surface of the earth. Much larger doses are required to retard excystment at this wavelength [8]. PR of wet cysts following exposure to this wavelength was also observed but as the data add nothing new to those reported for 265 nm they are not documented here.

Cysts need not be in aqueous medium during illumination to achieve PR. Those illuminated while in a closed dish over, but not immersed in, water or even over 8 M NaCl showed PR although the results were more variable than when they were in the balanced salt solution. Apparently sufficient hydration to activate the photoenzyme is all that is required for PR. Attempts to define the minimal relative humidity required for PR were not successful, partly because of the variability of the preparations.

It seemed of particular interest to determine whether PR would also occur in wet cysts exposed while dry to the space simulating non-ionizing radiation from an argon plasma arc lamp. Such radiation includes the entire span of wavelengths from the infra red to the vacuum UV, including the short wavelength visible and the near UV, both of which are active in PR. Illumination, in the wet state, of cysts so irradiated resulted in about as much PR as that observed in wet cysts irradiated with monochromatic 265 nm far UV radiation (Fig. 3).

3. Dark repair in irradiated cysts after hydration

Dark repair (DR) was not observed after irradiation of the dry cysts with radiation from an argon plasma arc, even after one month when the cysts were kept dry [10]. This was corroborated with dry cysts exposed to UV 265 nm. However, when the cysts irradiated for 30 seconds with the full spectrum of the argon plasma arc lamp filtered through the sapphire window were placed

in balanced salt solution, DR was found to occur to about the same degree as PR (Fig. 4), although the data are variable, perhaps because of the inconsistent handling encountered.

DISCUSSION

The fact that PR does not occur in dry cysts indicates that the photoenzyme necessary for PR is inactive in absence of water. Enzymatic activity generally increases with degree of hydration, even a relatively small amount of hydration permitting some activity [16]. It is possible that the small amount of PR (up to 20%) observed in early studies during the rainy season in "dry" cysts in equilibrium with room air occurred when the humidity of the air was adequate to activate the photoenzyme but the relative humidity of the room was not measured. A high degree of PR was observed in irradiated cysts put into suitable aqueous solutions during illumination. Even though oxygen consumption is difficult to detect in wet cysts [14, 15], the cysts are not without some metabolic activity because the time for excystment in yeast extract is reduced by prior immersion in water alone, suggesting activation of some enzymes by hydration. The kinetics of excystment suggest activation of enzymes as the first step in excystment [2], and the very factors necessary for excystment appear to be mostly prosthetic groups of enzyme systems or their precursors [7]. It thus seems likely that wetting alone sets into action some of the enzymes needed for excystment. In some species of protozoans (e.g., Euplotes taylori) found in saline environments dilution of the medium with water suffices to induce excystment [6]. This indicates that in some cryptobiotic organisms hydration alone initiates all the enzymatic activity necessary for the reorganization and reconstitution of the organelles of the vegetative form.

Inasmuch as effects of UV radiation on nucleic acids are subject to PR while those on protein are not [1], The finding of PR in Colpoda cysts suggests that far UV radiation retards excystment and reduces the population capable of excysting by virtue of action on nucleic acids or nucleoproteins. Since PR acts as a dose reduction [11], never being complete, far UV may have also affected molecules other than nucleic acids in the cysts, perhaps even producing some of the photoproducts found in spores of bacteria [5]. Unlike bacterial spore cysts do not at present lend themselves easily to harvesting in sufficient quantity for biochemical determinations. Dark repair of wet cysts to about the same extent as PR also suggests repair of nucleic acids damaged by UV since dark repair of other kinds of molecules has not been reported [17]. On the other hand, the relative completeness of dark repair makes it necessary to consider other possibilities.

While most of the experiments were carried out with wavelength 265 nm not present in sunlight reaching the surface of the earth but near the maximum action of far UV on cysts [8, 9], similar effects were observed at wavelength 320 nm which is represented in appreciable intensity in sunlight at the surface of the earth. However, a dose of almost ten times as much UV 320 nm must be delivered to the cysts to produce a delay comparable to UV 265 nm.

The greater vulnerability of wet than dry cysts has ecological interest. Considerable far UV is transmitted by clear (but not murky) fresh water; therefore, cysts in ponds may be reached by sunlight UV through many centimeters of water. However, the behavior of Colpoda (and probably most encysting ciliates) protects them from exposure to UV radiation. In general, just before encystment colpodas aggregate on and around detritus, primarily of plant origin. Thereby they are screened from UV radiation by the detritus and should some cysts still be exposed, the uppermost ones protect the lowest ones.

Cysts drifting in the air from place to place on earth are subject to sunlight damage. Therein their much greater resistance to UV in the dry state enhances their chance of survival. Yet when they fall into a pond or creek, both photoreversal and dark repair may undo some of the UV damage as they do for wet cysts in the water. The large degree of PR and DR may thus have considerable survival value.

PR and DR reverse not only much of the UV damage produced by sunlight reaching the surface of the earth, but also that following exposure to non-ionising space-simulating radiation. Should cysts survive a trip in space their survival would be enhanced by such capacity for recovery.

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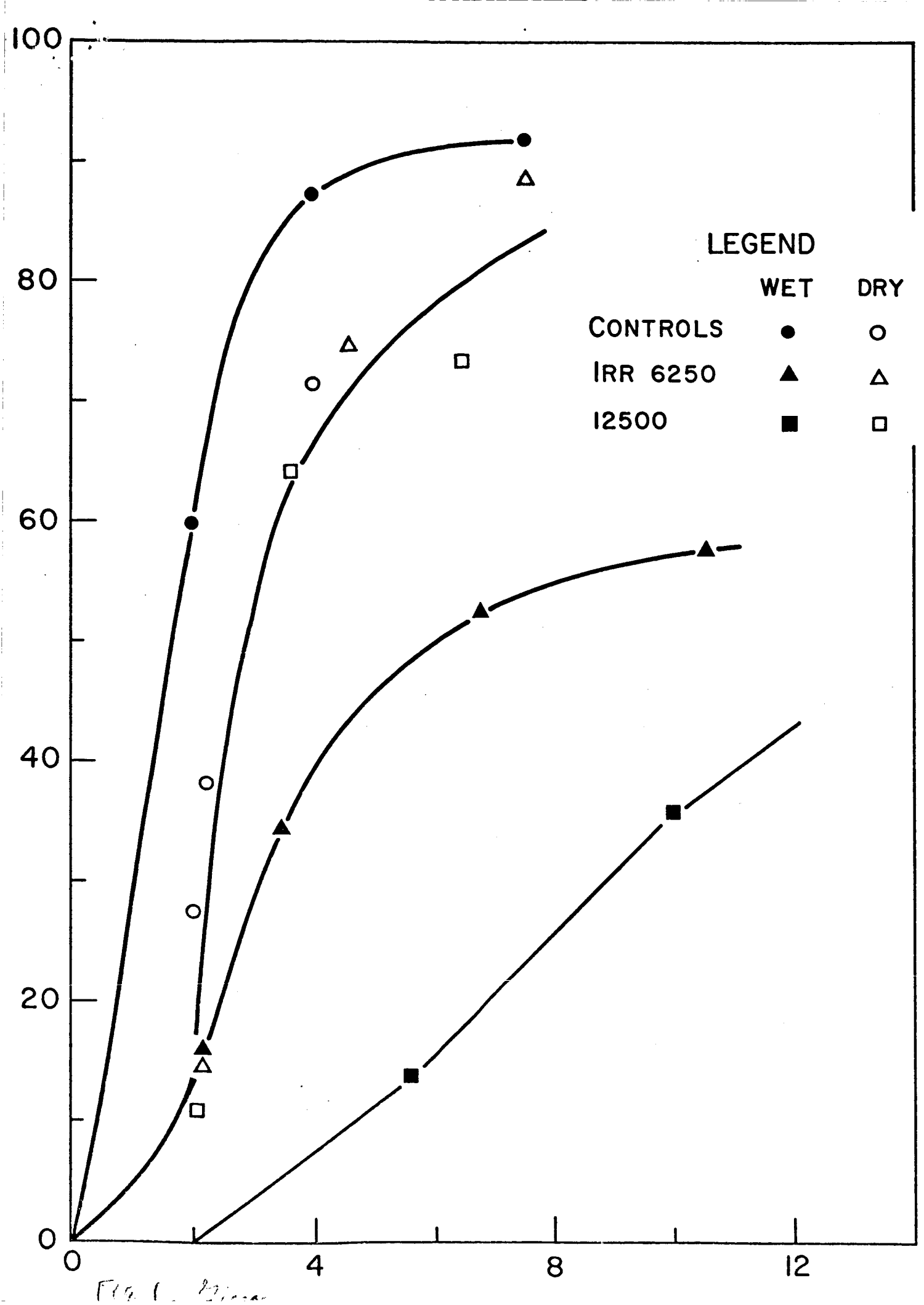
LEGENDS TO FIGURES

Fig. 1. Difference in effectiveness of small doses of 265 nm far UV radiation (triangles 6250 ergs mm^{-2}; squares, 12,500 ergs mm^{-2}) when delivered to wet (closed circles, triangles and squares) as compared to dry cysts (open circles, triangles and squares) in retarding excystment of Colpoda inflata cysts. Note that cysts (controls), which are kept in air that is of variable relative humidity and wetted just before being placed in excystment medium, show more rapid excystment than those kept dry over $CaCl_2$ until immersion in such medium. Ordinate, per cent excysted; abscissa, hours in excystment medium.

Fig. 2. Photoreactivation (PR) of 265 nm far UV-induced retardation of excystment in Colpoda cysts irradiated while wet with the following doses: triangles 6250 ergs mm^{-2}, squares, 12,500 ergs mm^{-2}, and diamonds, 25,000 ergs mm^{-2}; controls, circles. Open circles, triangles, squares and diamonds in light; closed circles, triangles, squares and diamonds in darkness. PR is measured by the difference in time for excystment of a certain fraction of cysts in darkness as compared to the time for excystment of the same fraction in the light. Ordinate, per cent excysted; abscissa, hours in excystment medium.

Fig. 3. Photoreactivation of UV-induced retardation of excystment in Colpoda cysts irradiated dry and then exposed to photoreactivating illumination in the wet state in one case (closed triangles, diamonds and squares), and in the dry state in the other (open triangles, diamonds and squares). Irradiation: triangles and diamonds, 50,000 ergs mm^{-2} 265 nm far UV; squares, 20 seconds argon plasma arc lamp radiation through a sapphire window (cutoff 145 nm). Ordinate, per cent excysted; abscissa, hours in excystment medium.

Fig. 4. Dark recovery (open squares, diamonds and triangles) of cysts exposed in dry state for 30 seconds irradiation to the argon plasma arc radiation filtered through the sapphire window, then placed in balanced salt solution which we changed once daily, and kept in darkness for the entire week. Those irradiated dry and kept dry in darkness for seven days are shown as solid squares, diamonds and triangles. The wet control, open circles. No dark recovery occurs in dry cysts. Ordinate, per cent excysted; abscissa, hours in excystment medium.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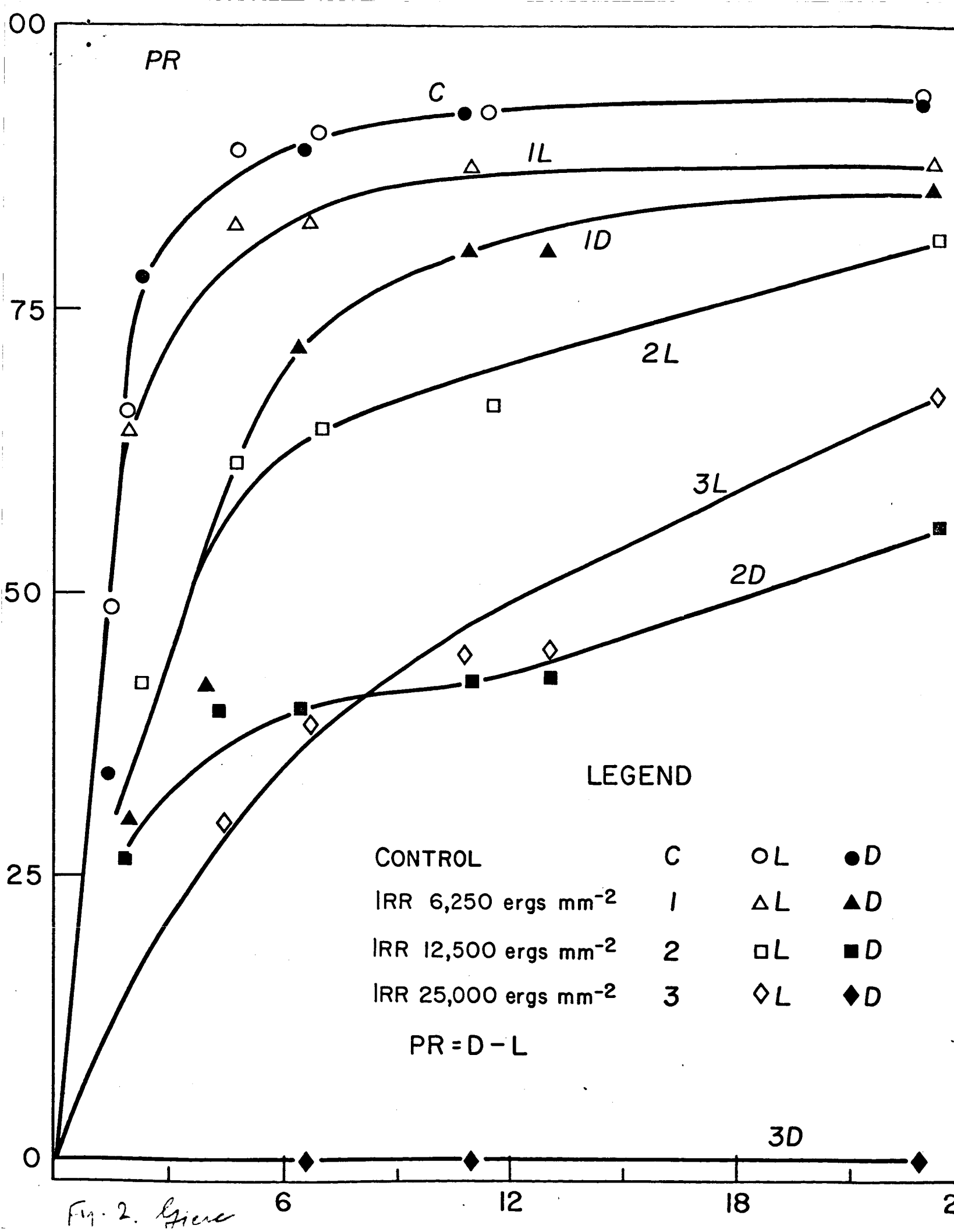
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IRR 25,000 ergs mm^{-2} 3 ◇L ◆D
PR = D − L

Fig. 2. Giese

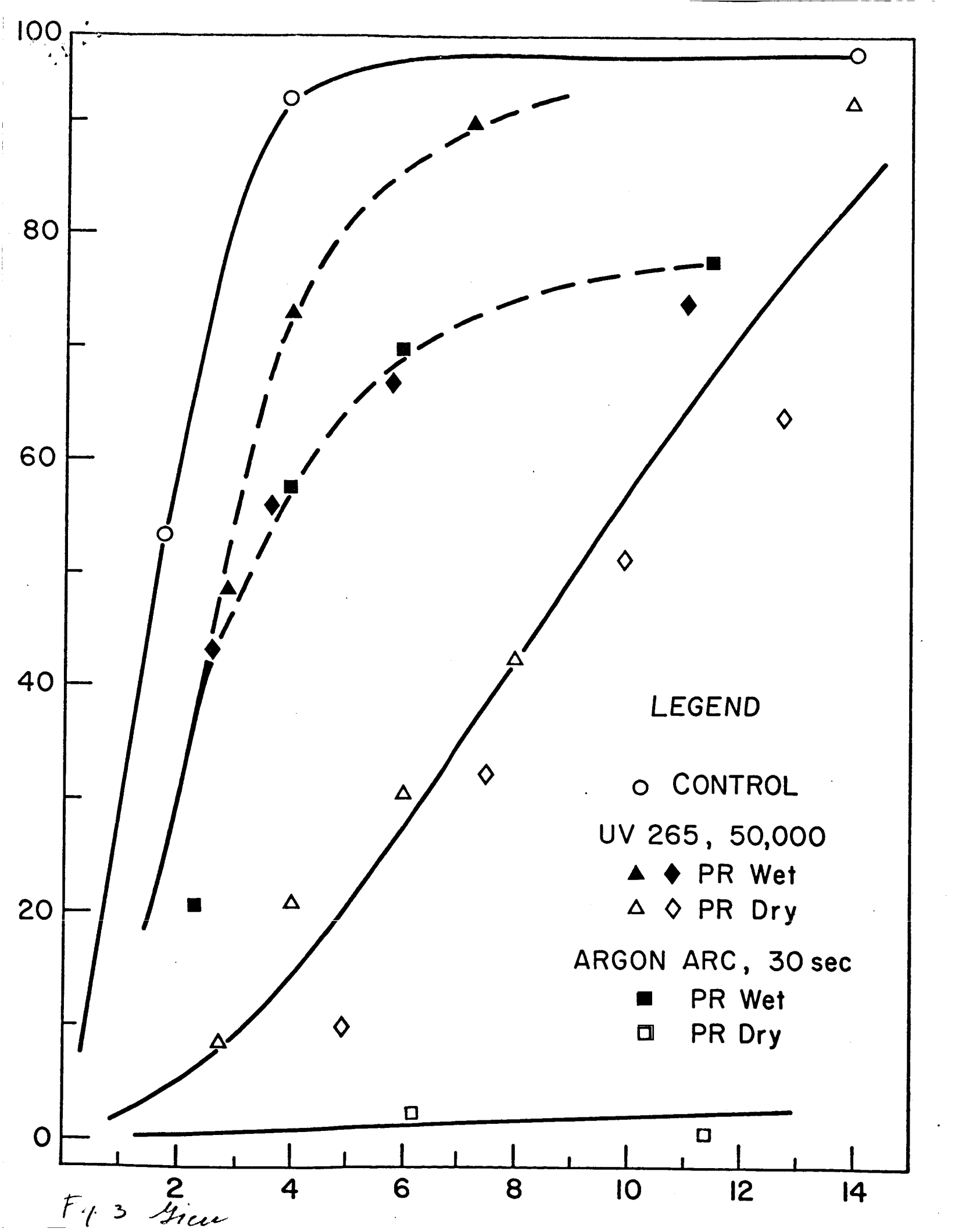
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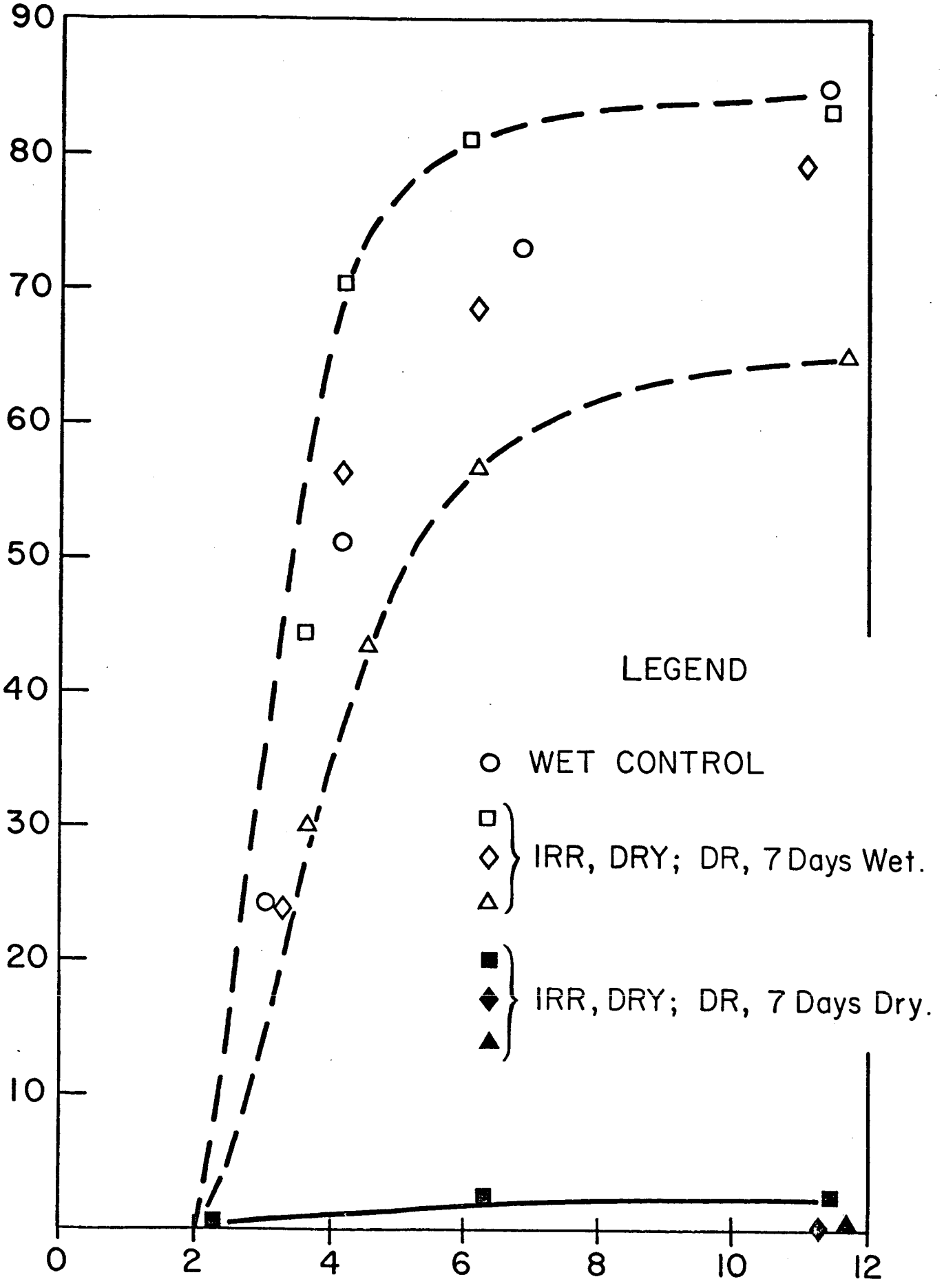
Fig 3 Given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LEGEND
WET CONTROL
IRR, DRY; DR, 7 Days Wet.
IRR, DRY; DR, 7 Days Dry.

Fig 4 Green